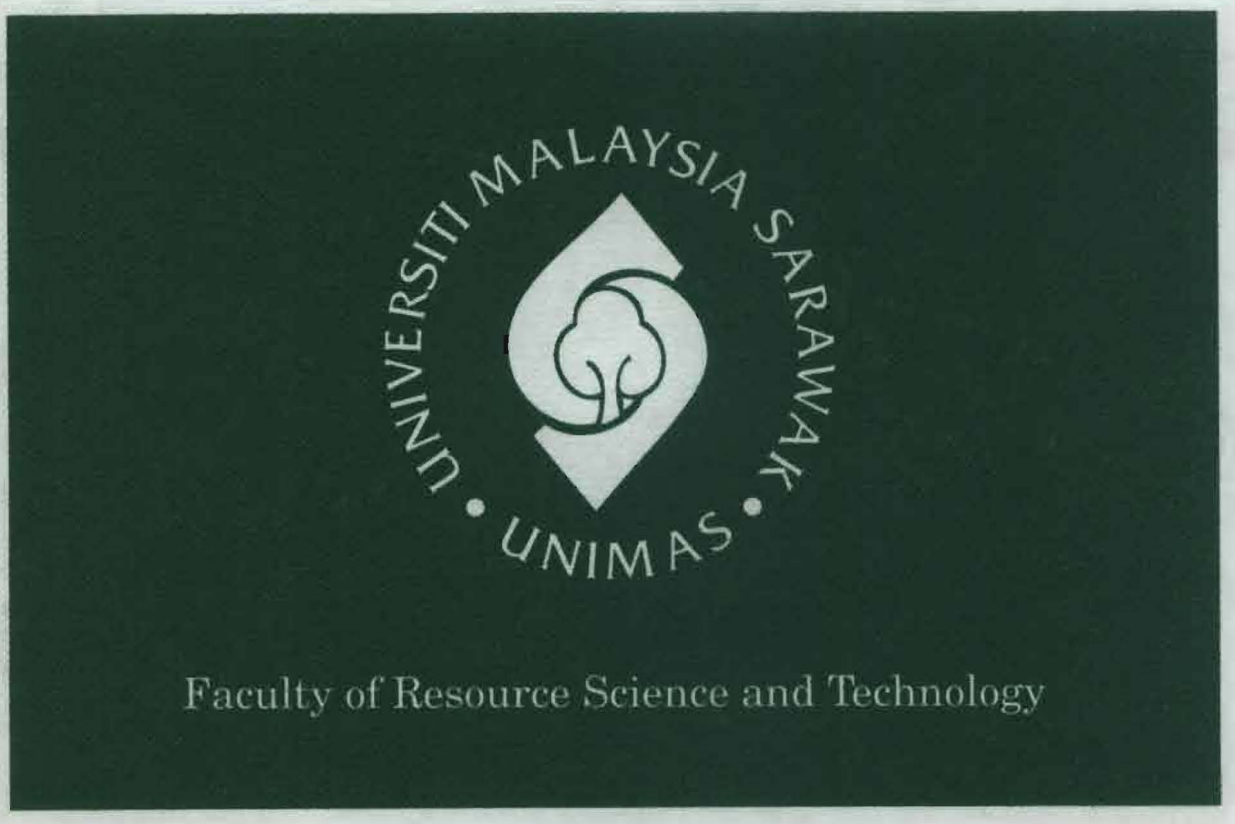

PROPAGATION OF KAPUR BUKIT (*Dryobalanops beccarii* DYER) THROUGH STEM CUTTING AND TISSUE CULTURE

REBICCA EDWARD @ MAY

Bachelor of Science with Honours
(Plant Resource Science and Management)
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This report is submitted in partial fulfillment of the requirements for the degree of
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DECLARATION

I hereby declare that no portion of the work referred to in this dissertation has been submitted in support of an application for another degree or qualification to this university or any other institution of higher learning.

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Propagation of Kapur Bukit (*Dryobalanops beccarii* Dyer) through Stem Cutting and Tissue Culture

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ABSTRACT

Kapur bukit (*Dryobalanops beccarii* Dyer) is a timber species of the Dipterocarpaceae. Kapur bukit is a good source of wood for construction purposes, particularly in plywood production. Vegetative propagation of this species through stem cutting and tissue culture was studied. In the study of vegetative propagation by stem cutting, rooting hormone were used and six treatments included were four different concentrations of indole-3-butyric acid (IBA), one with Seradix 2 and water as control. Three trials were conducted and the results showed that this species was difficult to be propagated by cuttings. An untreated cutting from the first trial developed root, shoot and new leaves, no cuttings rooted in second trial, while a cutting that has been treated with 2 g/l IBA from the third trial developed root, shoot and new leaf. For surface sterilization, the best regime for all explants was at the 20% 'Clorox' concentration for 15 minutes. The explants were cultured on soft ½ MS medium incorporated with 10 ml/l PPM + 10 ml/l Tetracycline. Best result for callus induction from shoot tips was observed in ½ MS medium supplemented with 1.0 ml PPM + 1.5 ml/l BAP. New growth of nodal segment was observed in ½ MS medium supplemented with 1.0 ml/l PPM + 1.0 ml/l BAP. Results from the study showed that further research is required to support these findings.

Key words: *Dryobalanops beccarii*, stem cutting, rooting hormone, surface sterilization, callus induction.

ABSTRAK

Kapur bukit (Dryobalanops beccarii Dyer) merupakan pokok balak famili Dipterocarpaceae. Ia merupakan sumber kayu yang baik untuk tujuan pembinaan, terutama sekali dalam penghasilan papan lapis. Kajian tentang propagasi vegetatif terhadap spesies ini dijalankan melalui kaedah keratan batang dan tisu kultur. Melalui kaedah keratan batang, hormon penggalak pengakaran digunakan dan terdapat enam rawatan: empat kepekatan asid indole-3-butyric (IBA) yang berbeza, satu dengan Seradix 2, and air sebagai kawalan. Tiga percubaan dijalankan dan keputusan menunjukkan bahawa spesies ini susah dibiakkan melalui keratan. Satu keratan dari percubaan pertama yang tidak dirawat mengeluarkan akar, pucuk dan daun, tidak terdapat keratan yang mengeluarkan akar dalam percubaan kedua dan untuk percubaan ketiga, satu keratan yang dirawat dengan 2 g/l IBA mengeluarkan akar, pucuk dan daun. Bagi pensterilan permukaan eksplan, rejim yang terbaik untuk mendapatkan semua eksplan yang bersih adalah pada 20% kepekatan 'Clorox' selama 15 minit. Kesemua eksplan dikultur pada media lembut ½ MS yang mengandungi 10 ml/l PPM + 10 ml/l Tetracycline. Keputusan yang terbaik untuk menginduksi kalus dari pucuk dihasilkan dalam media ½ MS yang mengandungi 1 ml PPM + 1.5 ml/l BAP. Manakala pertumbuhan segmen baru dari buku batang dihasilkan dalam media ½ MS yang mengandungi 1 ml PPM + 1.0 ml/l BAP. Melalui perolehan keputusan kajian ini, penyelidikan selanjutnya perlu dijalankan untuk menyokong keputusan yang telah diperolehi.

Kata kunci: Dryobalanops beccarii, keratan batang, hormon penggalak pengakaran, pensterilan permukaan, induksi kalus.

CHAPTER ONE

INTRODUCTION

Kapur bukit (*Dryobalanops beccarii* Dyer) is a timber species of the Dipterocarpaceae family. Also known as Keladan or Kapur keladan, the species is widespread in Mixed Dipterocarps Forest on clay-rich ridges and on yellow sandy soils derived from ferruginous sandstones. It is found throughout Sarawak (Anderson, 1980). Ashton (1996) reported that *D. beccarii* is only distributed within Borneo: Brunei Darussalam, Kalimantan, Sabah and Sarawak.

According to Browne (1955), *D. beccarii* is similar to *D. aromatica* and is of high quality and it is grouped in a medium weight kapur. It is found frequently in the most inaccessible forests. The wood and leaves are aromatic. Some of *Dryobalanops* sp. produces camphor oil, such as *D. aromatica* and *D. lanceolata* and the oil has been attributed medicinal properties. As for *D. beccarii*, it does not produces camphor oil.

Kapur bukit (*D. beccarii*) is a good source of wood for construction purposes, particularly in plywood production. It is used as furniture, joinery and packing cases, beams, columns, poles, mining timber, flooring, handles, toys and coffins. If properly treated with preservatives, it can be used for marine activities like ship-building, vehicle bodies, and railway sleepers. Other minor uses of the wood are chipboard and papermaking (Asean Regional Centre for Biodiversity Conservation, undated).

In a report made by Srivastava and Penguang Manggil (1981), the major problems in producing planting stock of desirable timber species in Malaysia are due to the irregular, unpredictable and short supply of seeds. This is due to irregular periodicity of flowering and fruiting in many important indigenous timber species. Other problems that should be considered are heavy insect and pest damage to seeds and lack or inadequacy of seed storage techniques and facilities. Therefore, for the purpose of establishing planted forest, it is necessary to develop vegetative propagation method, as fruiting and seed production in tropical forest species is irregular. Progenies derived from seeds may not be true-to-type. Breeding and selection for superior genotype take a long time to achieve. For tree improvement, if the 'plus tree' can be multiplied vegetatively for planting, the productivity can be increased faster as compared with seed propagation. Furthermore, vegetative propagation allows mass propagation of hybrids and polyploids that are having low sexual fertility (Bonga, 1977).

Though more difficult as compared to conventional propagation, cloning by means of tissue culture has many advantages. One is for the production of large quantity of planting material for reforestation programme and second is the rapid multiplication of selected clones in tree improvement programme. Genetic improvement of trees like any other plant species involves conventional selection and breeding as well as genetic engineering approach. Selection of tree with desired characteristics and planting the clonal material of the selected trees was the simplest approach. It can be carried out immediately by first identifying desired trees among trees in the existing population and then multiplied the 'plus' tree for planting. Tissue culture technique is the most obvious choice of method

for mass propagation of 'plus' trees. In genetic engineering approach, an *in vitro* regeneration system is useful as a pre-requisite technology. It is therefore useful to develop *in vitro* culture technique for tree improvement.

In a press report made on June 17th, 2004 by the Sarawak Tribune, the government of Sarawak has set a target of one million hectares of lands for reforestation for the next 15 years. This means planting 65 000 ha per year. Based on planting density of 800 trees per ha, there is a need to produce some 50 million trees per year for planting. From the same report, it is known that Kapur bukit has been identified as one of the indigenous species selected for planting as reforestation. Since fruiting of Kapur bukit is irregular, it is necessary to produce large quantity of planting material for the reforestation programme. Since there is no detail study yet on vegetative propagation of *D. beccarii* in Sarawak, the proposed study is initiated to develop methods for vegetative propagation of this species, by conventional as well as biotechnological approaches.

The objectives of this study are to find out if this species could be propagated by stem cutting and to attempt the development of the *in vitro* regeneration protocol for use in mass propagation of this species.

CHAPTER TWO

LITERATURE REVIEW

There are only a few publications on vegetative propagation of *Dryobalanops* species, such as *D. lanceolata* (Moura-Costa and Lundoh, undated); but there is none on *D. beccarii*. As for micropropagation, there is no report on tissue culture for *D. beccarii*, only on the species in the same family, such as *Shorea pinanga* and *Shorea macroptera* (Umboh, 1988 and Umboh *et al.*, undated).

2.1 Vegetative Propagation

According to Moura-Costa and Lundoh (undated), vegetative propagation by stem cutting has been investigated as a method of supplying planting material of dipterocarps. This statement is supported by many researchers which noted that vegetative propagation by cuttings can be used as an alternative to supply dipterocarp planting stock (Momose, 1978; Hallé and Hanif-Kamil, 1981; Srivastava and Penguang Manggil, 1981). Researchers reported that dipterocarps are considered difficult to root; therefore, results are sometimes unsatisfactory (Momose, 1978; Hallé and Hanif-Kamil, 1981; Srivastava and Penguang Manggil, 1981).

Halle *et al.* (1981) explained that through his personal communication with Salleh from Sarawak, stem cuttings of *Dryobalanops beccarii* and *Shorea albida* were

successfully rooted using a mixture of 0.2% concentration of naphthalene acetic acid (NAA) and 0.2% concentration of indole-3-butyric acid (IBA). The cuttings were taken from 6 months old seedlings. However, when cuttings from 18 months old seedlings were used, the results were not satisfactory.

2.1.1 Rooting of *Dryobalanops lanceolata* Burck (kapur paji) cuttings

Moura-Costa and Lundoh (undated) reported an experiment on the effects of three auxins (IBA, NAA and 2,4-D) at different concentrations on rooting of cuttings of *Dryobalanops lanceolata* Burck (kapur paji). Two-node cuttings were prepared using the apical portion of seedlings. The leaves are trimmed to approximately 30 cm^2 and the basal ends are dipped in a fungicide solution (0.1% w/v Benlate). In order to minimise stress, cuttings were placed into a mist chamber immediately after preparation. The three auxins that used were indole-3-butyric acid (IBA), a-naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D).

Auxins were prepared in the form of powder formulations by dissolving the pure compounds in 95% ethanol, mixed with talcum powder and dried at room temperature. The concentrations used were 0.2, 0.8 and 3.0% w/w in talcum powder and as for control; talcum powder was mixed with pure ethanol. Cuttings were rooted in a closed chamber mist propagation unit. After 12 weeks of the experiment, rooting of cuttings was observed and recorded.

From this experiment, they found that the control promoted the highest rooting percentage of the cuttings (83.3%); while among the plant growth regulator (PGR) treated cuttings, 0.2% of 2,4-D was the most effective in promoting rooting (72.2%). Nevertheless, higher concentrations of 2,4-D (0.8 and 3.0%) decreased rooting percentages and caused desiccation of some cuttings. As a result, this experiment showed that the rooting for *D. lanceolata* cuttings did not require exogenous auxins since auxins had a suppressant effect on rooting of *D. lanceolata*.

Consequently, after the previous study, Moura-Costa (1996) conducted a similar rooting experiment for the cuttings of *D. lanceolata*. For this latter study, no auxins were used since earlier experiments showed that IBA, NAA and 2,4-D (at 0.2, 0.8 and 3.0% w/w) suppressed rooting of juvenile cuttings of some dipterocarps. Cuttings were rooted in a mist propagator unit covered by a transparent plastic sheet to increase relative humidity. After 12 weeks, rooting percentage was around 87 % (n=396, SE=1.6) for *D. lanceolata* and 65 % (n=40, SE=2.4) for *Shorea* spp. After roots formed, the cuttings were potted and kept in plastic covered chambers in the shade house for two weeks during the acclimatization stage, before exposure to normal nursery conditions.

2.2 Micropropagation (Tissue Culture)

Tissue culture is an alternative to the conventional methods, and has been successfully employed to produce true-to-type propagules. It can provide a means of altering the developmental phase of old and mature trees of some difficult-to-root species. Besides that, mature plant tissues of explants taken for micropropagation undergo a partial rejuvenation process, though this induced rejuvenation may be temporary. Such rejuvenated plant material is physiologically different from its former mature status and often shows a restored rooting ability (Moura-Costa, 1996).

Luukkanen (2001) acknowledged that in tropical broadleaved tree species, the practical applications of micropropagation seem to concentrate on tissue culture, while propagation using somatic embryogenesis seems to be more promising in (mostly temperate-zone) conifer propagation. Both groups of techniques require exact protocols for each species, however in several tropical forest tree species, practical applications are already available. Forestry-related use of micropropagation for organogenesis has been successfully developed at least in the following genera: *Acacia, Bombax, Casuarina, Dalbergia, Eucalyptus, Ficus, Gmelina, Populus, Platanus, Salix, Shorea, Tectona and Terminalia.*

It has been reported (Sudarmonowati, 2000) that the availability of good quality planting materials of forest tree species is the main problem in the tropical countries that encounter high rate of deforestation. In order to solve this problem, rapid propagation

techniques such as tissue culture or *in vitro* propagation that are able to provide a large number of uniform planting materials are prerequisite. Unlike other plant species, the progress of *in vitro* propagation of tropical forest trees has been relatively slow as it is hindered by high content of phenolic compounds. The number of the trees that could be propagated *in vitro* is very limited. Most technique used is shoot multiplication as somatic embryogenesis is very much influenced by many factors. *Acacia mangium, Eucalyptus urophylla* and *Tectona grandis* are among the species that have been propagated. On a large scale, basis and some of them are still being tested in the field. Embryogenic callus had been produced from certain species such as *Shorea pinanga* and *Pometia pinnata.*

2.2.1 Development of pre-somatic embryogenesis on *Shorea pinanga*

Umboh (1988) reported that induction of multiple shoots of *Shorea pinanga* can only be obtained from the embryo culture, and there is no response from other types of explants such as shoot tips, stem nodes and axillary buds. A basal MS (Murashige and Skoog, 1962) medium supplemented with glutamine (400 mgl^{-1}), 2,4-D (1 to 10 mgl^{-1}) and a range of 500, 1000 and 1650 mgl^{-1} ammonium nitrate was the media which callus was induced. Observation on the production of callus was made in the medium MS with normal NH_4NO_3 (1650 mgl^{-1}) added with glutamine (400 mgl^{-1}) to 2,4-D (10 mgl^{-1}). The formation of globular structures is greater as the concentration of 2,4-D is higher in the basal medium.

The globular masses are subcultured from the induction medium to a half MS medium. After six months, they developed and became heterogeneous, bigger, and more compact in dark green colour. Some of them were light brown and others were green, which separated from the colonies and formed to egg-shaped masses. The egg-shaped structures were then cultured on basal MS medium with activated charcoal (5 grl^{-1}). After a month, the egg-shaped structures formed into the initial somatic embryo.

2.2.2 Somatic embryogenesis of *Shorea macroptera*

As recorded by Umboh *et al.*, (undated), *Shorea* spp are difficult to regenerate naturally as well as conventional propagation. For that reason, sixty zygotic embryos of immature fruits of *S. macroptera* were induced in the same induction media of *S. pinanga* and *S. stenoptera* (Umboh, 1988) which composed of half strength and full MS supplemented with 2,4-D of 0, 5 and 10 mg/l, glutamine 100 mg/l and glucose 30 g/l. The direct embryoid cells developed after 12 weeks in a simple basal medium of MS. Two phases have been tried to maintain and multiply the cells into the development of viable globular formations in three different media: MS + 2,4-D 1 mg/l, WPM + BAP 1 mg/l, and WPM without growth regulator. The development of embryo-like callus was also obtained in three different basal media without growth regulator: the *Pinus merkusii* induction medium (Umboh, 1988); WPM basal medium, and the half-strength of MS. The normal regeneration of embryoid cells into plantlet formation was observed.

2.2.3 Browning exudates from explants

Bonga and Von Aderkas (1992) reported that toxic phenolic exudates cause injury, which creates more exudates and thus more browning. Some of the methods that they suggested are by using charcoal to bind exudates and to do frequent subculture. One of the advantages by subculturing frequently is that the parts of the tissue can be removed before the healthy cells are overwhelmed with their toxic phenolics.

CHAPTER THREE

MATERIALS AND METHODS

In this study, two methods of propagation were carried out. They were vegetative propagation by stem cutting and micropropagation with the application of tissue culture techniques.

Study Site

Vegetative propagation by stem cutting was conducted in the Greenhouse, while micropropagation was carried out at the Plant Tissue Culture Laboratory of Faculty of Resource Science and Technology, University Malaysia Sarawak.

Resource of Study

Sarawak Forest Corporation (SFC) from their nursery at Semengoh, Sarawak, supplied stock plants of *Dryobalanops beccarii*. The staff at the nursery maintained the stock plants. There were 300 seedlings grown in poly-bags containing forest topsoil. The age of seedlings was approximately two years.

Experimental Design

For experiment on vegetative propagation by stem cutting, the experimental design was Completely Randomized Design (CRD). There were 6 treatments with 10 replicates per treatment. As for micropropagation, it is a two-factor factorial experiment with 3 replicates

per treatment combination arranged in a CRD. It was done with three levels of 'Clorox' concentrations applied at four exposure times (3 x 4 factorial) and 3 replicates per treatment for each type of explants.

Statistical Analysis

The results were analysed statistically by using the Univariate analysis of variance (ANOVA): Two-way analysis of variance with replication. While Least Significant Different (Fisher's LSD) was used to test the means. The results obtained were analyzed using the SPSS statistical package version 11.5.

3.1 Vegetative Propagation by Stem Cutting

3.1.1 Preparation of sand bed and plant growth regulator

River sand was used as the rooting medium and the sand bed was watered thoroughly; three days before rooting experiment. Transparent polythene sheet was fixed on the sides of the sand bed's frame and roof, which were always kept moist, in order to create high humidity by reducing air movement and rate of evaporation. The plant growth regulator (PGR) solutions were prepared the day before the experiment.

3.1.2 Preparation and treatment of cuttings

Cuttings were taken from the SFC nursery in the morning. The cut at the basal end was made oblique to ensure greater surface for absorption. Clipping was

done with a sharp pruning shear to minimize tissue damage. To prevent from drying, the cuttings were wrapped with wet newspaper immediately after cutting and brought back to UNIMAS plant shed for rooting.

The lower part 2 to 3 cm of the cuttings were immersed into the PGR solution for 1 minute; air dry for half an hour and then ⅓ of the cuttings was inserted into the sand bed. The cuttings were randomly distributed by mean of CRD into the sand bed. After insertion, the medium was pressed firmly around the cuttings. Watering was carried out immediately to create high humidity around the cuttings and to ensure an adequate amount of moisture in the rooting zone.

3.1.3 Watering schedule

For the first two weeks after planting, cuttings were sprayed with hand-held sprayer for five minutes in every three hours between 8.00 am to 5.00 pm. During the rest of the experimental period, manually controlled misting heads was used and the cuttings were watered for five minutes, three times a day.

Three trials were conducted for the experiment; each trial was carried out after the earlier trial was terminated.

3.1.4 First Trial

Six treatments were carried out: four with IBA (indole-3-butyric acid) at different concentrations i.e. 1, 2, 3 and 4 g/l, one with Seradix 2 (commercial rooting powder) and one with water as a control.

Three to four-node cutting were made and the cuttings were taken from green turning brown section downwards from the main stem. All the leaves at the lower three nodes were trimmed off. One leaf on the top node was retained. There were a total of 90 cuttings; with 15 cuttings for each treatment.

3.1.5 Second Trial

Six treatments were carried out: two with IBA at two different concentrations, i.e. 0.2% and 0.8% w/w in talcum powder, two with 2,4-dichlorophenoxyacetic acid (2,4-D) at two different concentrations, i.e. 0.1% and 0.2% w/w in talcum powder, one with Seradix 2 and one with talcum powder mixed with pure ethanol was used as a control. Powder formulations were prepared by dissolving the pure compounds (Sigma Chemical Company Ltd., UK) in 95% ethanol, mixing the solution with talcum powder and allowing it to dry at room temperature.

Two-node cuttings; 7 to 10 cm length, were taken from the apical shoots of seedlings and their leaves were trimmed off. There were a total of 90 cuttings; with 15 cuttings for each treatment.

3.1.6 Third Trial

Due to limited material, the third trial only consisted of three treatments. The treatments were as follow: 2 g/l IBA, Seradix 2 and water as a control.